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TI Comparison of the substrate specificity of two potyvirus  
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AB The substrate specificity of the nuclear inclusion protein a  
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proteolytic enzymes from two potyviruses, the tobacco etch virus  
(TEV) and  
tobacco vein mottling virus (TVMV), was compared using  
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substrates. Mutations were introduced into TEV protease in an effort to identify key determinants of substrate specificity. The specificity of the mutant enzymes was assessed by using peptides with complementary substitutions. The crystal structure of TEV protease and a homology model of TMV protease were used to interpret the kinetic data. A comparison of the two structures and the experimental data suggested that the differences in the specificity of the two enzymes may be mainly due to the variation in their S4 and S3 binding subsites. Two key residues predicted to be important for these differences were replaced in TEV protease with the corresponding residues of TMV protease. Kinetic analyses of the mutants confirmed that these residues play a role in the specificity of the two enzymes. Additional residues in the substrate-binding subsites of TEV protease were also mutated in an effort to alter the specificity of the enzyme.

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 DN PubMed ID: 2475971  
 TI Characterization of the catalytic residues of the tobacco etch virus 49-kDa proteinase.  
 AU Dougherty W G; Parks T D; Cary S M; Bazan J F; Fletterick R J  
 CS Department of Microbiology, Oregon State University, Corvallis 97331-3804.  
 NC DK39304 (NIDDK)  
 SO Virology, (1989 Sep) Vol. 172, No. 1, pp. 302-10.  
 Journal code: 0110674. ISSN: 0042-6822.  
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 AB The 49-kDa proteinase of tobacco etch virus (TEV) cleaves the polyprotein

derived from the TEV genomic RNA at five locations. Molecular genetic and biochemical analyses of the 49-kDa TEV proteinase were performed to test its homology to the cellular trypsin-like serine proteases. A cDNA fragment, containing the TEV 49-kDa proteinase gene and flanking sequences, was expressed in a cell-free transcription/translation system and resulted in the formation of a polyprotein precursor that underwent rapid self-processing. Site-directed mutagenesis was used to test the effect of altering individual 49-kDa amino acid residues on proteolysis. The data suggest that the catalytic triad of the TEV 49-kDa proteinase could be composed of the His234, Asp269, and Cys339. These findings are consistent with the hypothesis that the TEV 49-kDa proteinase is structurally similar to the family of serine proteinases with the substitution of Cys339 as the active site nucleophile. A structural model of the TEV 49-kDa proteinase proposes other virus-specific differences in the vicinity of the active site triad and substrate-binding pocket. The structure may explain the observed negligible effect of most cellular proteinase inhibitors on the activity of this viral proteinase.

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Department of Microbiology, Oregon State University, Corvallis 97331-3804.

The 49-kDa proteinase of tobacco etch virus (TEV) cleaves the polyprotein derived from the TEV genomic RNA at five locations. Molecular genetic and biochemical analyses of the 49-kDa TEV proteinase were performed to test its homology to the cellular trypsin-like serine proteases. A cDNA fragment, containing the TEV 49-kDa proteinase gene and flanking sequences, was expressed in a cell-free transcription/translation system and resulted in the formation of a polyprotein precursor that underwent rapid self-processing. Site-directed mutagenesis was used to test the effect of altering individual 49-kDa amino acid residues on proteolysis. The data suggest that the catalytic triad of the TEV 49-kDa proteinase could be composed of the His234, Asp269, and Cys339. These findings are consistent with the hypothesis that the TEV 49-kDa proteinase is structurally similar to the trypsin-like family of serine proteinases with the substitution of Cys339 as the active site nucleophile. A structural model of the TEV 49-kDa proteinase proposes other virus-specific differences in the vicinity of the active site triad and substrate-binding pocket. The structure may explain the observed negligible effect of most cellular proteinase inhibitors on the activity of this viral proteinase.

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